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14. ABSTRACT
Purpose: The goal of this project is to determine the underlying synaptic dysfunction in Tuberous Sclerosis Complex (TSC). Scope: TSC is a multi-system genetic disorder with central nervous system dysfunction as a defining factor. The most common clinical features are mental retardation, epilepsy, autism, anxiety and mood disorders. Fragile X syndrome (FXS), another form of inherited mental retardation and autism, shares many of the same molecular and clinical features. Much of the pathophysiology in FXS can be ameliorated through modulation of Group 1 metabotropic glutamate receptors (mGluRs). Since both disorders share key features suggests that they may also share common pathogenic mechanisms. Therefore, determined whether altered synaptic protein synthesis, plasticity and hippocampal-dependent behavior could be ameliorated through modulation of mGluR's in a mouse model of TSC. Major findings: Unlike in FXS where negative modulation of mGluR's has proven beneficial, we found that augmenting mGluR function either genetically or through application of an mGluR5 positive allosteric modulator (PAM) ameliorates several of the synaptic and hippocampal-dependent behavioral deficits observed in a mouse model of TSC. Significance: These results suggest that modulation of mGluR activity with PAMs may be a therapeutic intervention for several of the deficits observed in TSC.

15. SUBJECT TERMS
autism, Tuberous Sclerosis Complex, Fragile X Syndrome, metabotropic glutamate receptor, rapamycin, positive allosteric modulator, long-term depression, synaptic protein synthesis

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Introduction:

Tuberous sclerosis complex (TSC) is a multi-system genetic disorder that affects roughly 1 in every 6000 individuals. The disorder is characterized by benign tumors in multiple organs, including the brain. Central nervous system dysfunction is a defining factor in the disorder, with the some of the most common clinical features being mental retardation, epilepsy, autism, anxiety and mood disorders (Prather & de Vries, 2004). Fragile X syndrome (FXS), another form of inherited mental retardation and autism, shares many of the same clinical features as TSC. Moreover, there are biochemical interactions between FMRP, the protein disrupted in FXS, and the TSC1/TSC2 complex (Narayanan, et al., 2007). A leading theory on the etiology of FXS holds that exaggerated protein synthesis linked to activation of Gp1 mGluRs (mGluRs 1 and 5) results in changes in synaptic structure and function that are the root cause of cognitive impairment. Indeed, genetic reduction and pharmacological inhibition of mGluR5 has been shown to correct almost every phenotype examined in the *Fmr1* KO mouse (Dolen, et al., 2007; Michalon, et al., 2012). That there are commonalities between TSC and FXS, ranging from the molecular to clinical level, suggests that TSC and FXS may share common pathogenic mechanisms. This inspired us to perform the complimentary experiments used to characterize FXS in a well-established animal model of TSC, the $Tsc2^{+/-}$ mouse. Unexpectedly, we found a reduction in mGluR-dependent LTD and protein synthesis in the hippocampus of the Tsc2+/- mice. We hypothesize that these alterations in mGluR dependent plasticity and protein synthesis are causally related to the cognitive dysfunctions seen in TSC and that *augmenting* mGluR5 function with mGluR5 positive allosteric modulators (PAMs) will ameliorate the synaptic and behavioral deficits seen in $Tsc2^{+/-}$ mice. Corroborating the efficacy of mGluR5 PAM treatment at the behavioral level is an essential step in validating this intervention as a putative therapy for TSC. Therefore, we propose to fully characterize synaptic and behavioral impairments in $Tsc2^{+/-}$ mice and to determine the effectiveness of mGluR PAM treatment on these deficits. The specific aims of this proposal are:

<u>Specific aim 1:</u> Comparison of mGluR5 PAM and rapamycin treatment on hippocampal protein synthesis and hippocampal dependent behavioral deficits in $Tsc2^{+/-}$ mice.

<u>Specific aim 2:</u> Characterization of visual cortical plasticity in $Tsc2^{+/-}$ mice and comparison of mGluR5 PAM and rapamycin treatment.

<u>Specific aim 3:</u> Characterization of higher cognitive function in $Tsc2^{+/-}$ mice.

Body:

Since the initiation of this project on April 1 2011, we have made considerable progress in the proposed research. We have completed the majority of experiments outlined in the first and third Aims of our approved Statement of Work and have initiated the experiments proposed in Aim 2. The progress made on this project to date has culminated in numerous presentations at scientific meetings and a peer-reviewed article describing our findings has been published in the journal *Nature* (Auerbach, Osterweil, & Bear, 2011).

Specific aim 1: Comparison of mGluR5 PAM and rapamycin treatment on hippocampal protein synthesis and hippocampal dependent behavioral deficits in $Tsc2^{+/-}$ mice.

As addressed in our 2012 progress report, specific Aim 1 was completed. In the experiments proposed under this aim, we directly compared the effect of rapamycin and mGluR5 PAM treatments on hippocampal protein synthesis and determined the extent to which mGluR5 PAM treatment can reverse the behavioral impairments previously shown to be rescued by rapamycin in $Tsc2^{+/-}$ mice (Ehninger, et al., 2008).

In *Fmr1* KO mice, it is believed that this excessive protein synthesis serves as the cellular mechanism which leads to the exaggeration of mGluR mediated processes, including mGluR-LTD (Bear, Dolen, Osterweil, & Nagarajan, 2008). Therefore, we hypothesized that decreased basal protein synthesis levels may account for the deficient mGluR-LTD seen in $Tsc2^{+/-}$ mice and, as predicted, basal protein synthesis rates are in fact reduced in $Tsc2^{+/-}$ mice. This finding suggested the possibility that protein(s) required for mGluR-LTD are deficiently translated in the hippocampus of $Tsc2^{+/-}$ mice. Additional experiments were performed examining the levels of Arc, a plasticity-related protein that is rapidly synthesized in response to mGluR activation and is required for mGluR-LTD (Park, et al., 2008; Waung, Pfeiffer, Nosyreva, Ronesi, & Huber, 2008). Arc expression was indeed decreased in $Tsc2^{+/-}$ hippocampal slices and was found to be due to a significant reduction in Arc translation in the hippocampus of $Tsc2^{+/-}$ mice. These results strongly suggest that mGluR-LTD is deficient in the $Tsc2^{+/-}$ hippocampus because of a decrease in the translation of proteins required to stabilize LTD, including Arc.

To test the hypothesis that the deficient basal protein synthesis observed in $Tsc2^{+/-}$ mice is a specific consequence of unregulated mTOR activity, we first examined the effects of the mTORC1 inhibitor rapamycin. We found that acute rapamycin treatment (20 nM) restored protein synthesis levels in the $Tsc2^{+/-}$ mice to WT levels, whereas this same treatment had no effect on slices from WT mice. These findings are entirely consistent with our previous observations demonstrating that acute rapamycin treatment restores mGluR-LTD in $Tsc2^{+/-}$ mice to WT levels, whereas this same treatment has no effect on mGluR-LTD in slices from WT mice. Moreover, the rescue of mGluR-LTD in $Tsc2^{+/-}$ mice appears to be specifically due to recovery of the protein-synthesis-dependent component of mGluR-LTD, as the effect of rapamycin in $Tsc2^{+/-}$ slices is eliminated in the presence of the protein synthesis inhibitor cycloheximide.

A model that best fits these data is that unregulated mTOR activity caused by the $Tsc2^{+/-}$ mutation suppresses the protein synthesis that is required for mGluR-LTD. In the mouse model of FXS, excessive mGluR-LTD and hippocampal protein synthesis can be corrected by reducing signaling through mGluR5 activation (Dolen, et al., 2007; Osterweil, Krueger, Reinhold, & Bear, 2010). Therefore we determined if the opposite approach of potentiating mGluR5 signaling with a positive allosteric modulator (PAM) could be beneficial in this model of TSC. Indeed, we found that pretreatment of hippocampal slices with the mGluR5 PAM 3-cyano-N-(1,3-diphenyl-1H-pyrazol-5-yl)benzamide (CDPPB;(Kinney, et al., 2005)) restored the magnitude of mGluR-LTD in *Tsc*$2^{+/-}$ mice to WT levels. The rescue of LTD appears to be due specifically to recovery

of the protein-synthesis-dependent component because the effect of CDPPB was eliminated by cycloheximide. Consistent with this conclusion, CDPPB treatment also restored basal protein synthesis levels and rescued the deficit in Arc synthesis in the $Tsc2^{+/-}$ mice. Thus, allosteric augmentation of mGluR5 signaling can overcome the inhibitory effect of unregulated mTOR activity on the synaptic protein synthesis that supports LTD.

We have shown that there is disrupted mGluR function in the hippocampus of $Tsc2^{+/-}$ mice. The hippocampus is an area of the brain known to be vital for many forms of learning and memory (Eichenbaum, 2004). Alterations in hippocampal function have adverse effects on learning and cognition, and therefore are likely to contribute to the cognitive impairments seen in TSC. Therefore, it was imperative to determine if mGluR5 PAM treatment is effective at reversing hippocampal-dependent learning deficits in $Tsc2^{+/-}$ mice as previously it has been demonstrated that rapamycin treatment reverses the deficits in these mice (Ehninger, et al., 2008). Therefore, we characterized the nature of the relationship between mTOR signaling, mGluR dependent plasticity, and the electrophysiological and behavioral impairments observed in $Tsc2^{+/-}$ mice. Under this aim, we determined if mGluR5 PAM treatment of $Tsc2^{+/-}$ mice could reverse the behavioral impairments previously shown to be rescued by rapamycin.

Enhinger et al. (2008) reported an impairment in the ability of $Tsc2^{+/-}$ mice to distinguish between familiar and novel contexts in a fear conditioning task. This task is hippocampus dependent (Frankland, Cestari, Filipkowski, McDonald, & Silva, 1998), and requires both mGluR5 activation (Lu, et al., 1997) and new protein synthesis at the time of training (Steidl & Yool, 1999). First we confirmed that WT mice could discriminate familiar from novel contexts and the quality of this behavior was comparable to that previously reported. However, unlike the WT animals, $Tsc2^{+/-}$ mice did not demonstrate the ability to discriminate between the familiar and novel contexts as they spent a comparable amount of time freezing in both contexts (Auerbach, et al., 2011). These findings demonstrated that this behavioral assay is a sensitive measure of disruptions of a hippocampal-dependent process.

Next we determined the effect of mGluR5 PAM treatment on the context discrimination deficits observed in $Tsc2^{+/-}$ mice. To test the effect of augmenting mGluR5 signaling, WT and $Tsc2^{+/-}$ mice were injected intraperitoneally with CDPPB (10 mg/kg^{-1}) 30 min before training. Although this treatment had no effect on behavioral performance in WT mice, it was sufficient to correct the deficit in context discrimination observed in $Tsc2^{+/-}$ mice. These results show that augmentation of mGluR5 signaling is beneficial at the behavioral level in $Tsc2^{+/-}$ mice and that disrupted mGluR5 function may be relevant to cognitive impairments associated with TSC.

These data demonstrate that mutations causing FXS and TSC, two disorders associated with autism and intellectual disability, show mirror symmetrical alterations in protein-synthesis-dependent LTD and have beneficial responses to treatments that modulate mGluR5 in opposite directions. Since these two mutations cause opposite alterations in mGluR-LTD and protein synthesis, this raised the intriguing possibility that these two mutations could cancel one another on this functional axis. We tested this hypothesis by performing an additional series of experiments, where we introduced an Fmr1 deletion into the $Tsc2^{+/-}$ background by crossing $Tsc2^{+/-}$ males with $Fmr1^{+/-}$ females. This approach enabled us to compare directly with WT the effects of the $Tsc2^{+/-}$ and $Fmr1^{-/}$y mutations in littermates reared under identical conditions. As expected, when we compared context discrimination in the $Tsc2^{+/-}$ and $Fmr1^{-/}$y mice we found that that they both share a deficit in this measure of memory. Remarkably, this memory deficit was erased in the double mutants. In an additional set of electrophysiological experiments we tested whether a similar rescue of mGluR-LTD was observed. We found that mGluR-LTD was diminished in $Tsc2^{+/-}$ mice and excessive in the $Fmr1^{-/}$y mice, compared with WT. However, mice harboring both mutations showed mGluR-LTD that was indistinguishable from WT. These results demonstrate that the opposing synaptic deviations seen in $Tsc2^{+/-}$ and $Fmr1^{-/}$y mice manifest similarly at the behavioral level, and introducing both mutations not only reverses the disruptions of synaptic plasticity but rescues memory impairment as well.

Having demonstrated a robust and reversible phenotype in an assay of hippocampal-dependent function using the context discrimination paradigm, we chose not to pursue an investigation determining the effects of mGluR5 PAM treatment on Morris water maze performance as little additional insight would be gained. We instead focused our attention on characterizing higher cognitive function in $Tsc2^{+/-}$ mice during our second year of funding (see Aim 3 below).

Specific aim 3: Characterization of higher cognitive function in $Tsc2^{+/-}$ mice.

The cognitive manifestations of TSC are complex and diverse. However, the cognitive impairments often associated with this disorder are deficits in executive-attentional function (Gillberg, Gillberg, & Ahlsen, 1994; Prather & de Vries, 2004). Executive function is an umbrella term used to describe a set of cognitive skills involved in attention, inhibitory control and cognitive flexibility. Despite extensive clinical research into this core deficit in TSC, impairments in executive function have received little attention in pre-clinical research. In particular, there has been no published research exploring executive function in animal models of TSC. Under this aim we proposed to establish a battery of behavioral measures to assess executive function in Tsc2+/- mice, and to subsequently utilize this battery to investigate the role of metabotropic glutamate receptor signaling if a deficit was observed.

Having demonstrated that the hippocampal-dependent deficit in contextual fear conditioning is ameliorated by both pharmacological facilitation of mGlur5 function with PAMs and genetic intervention, we focused our efforts during this reporting period on determining whether higher cognitve functions are also compromised in the Tsc2+/- mouse. To assess these processes we utilized the five choice serial reaction time task (5CSRTT), which is a well established operant conditioning paradigm used to assess executive function in rodents (Krueger & Bear, 2011). Firstly, we successfully initiated a partial food deprivation paradigm in both WT and Tsc2+/- mice and found no apparent adverse effects in either group of animals. Both groups of animals then underwent daily habituation sessions in the behavioral testing apparatus (see Figure 1). The 5CSRTT uses an operant test chamber equipped with five nose-poke holes on one wall, i.e., apertures that can detect a nose-poke response by the mouse, and a food magazine on the opposing wall into which a food reward can be delivered. In these experiments, Tsc2+/- mice and WT littermates were trained to perform a nose-poke response in an illuminated aperture in order to obtain a food reward (see (Krueger & Bear, 2011)for details of the training phases). In the first phase, all five apertures were illuminated and active, and a nose-poke response in any of the apertures resulted in the delivery of a food reward. Similar to what has been documented in FXS mice (Krueger & Bear, 2011), we found that Tsc2+/- mice showed no significant difference in the number of days taken to acquire this task as compared to WT littermates, suggesting that they are not impaired in the acquisition of an appetitive instrumental response under the current experimental conditions. In the second phase, only one of the five apertures was illuminated (see Figure 2, top panel), and only a nose-poke response in this single aperture was rewarded. Whereas FXS model mice have been shown to take more days and commit more errors while reaching criterion in this phase of the task (see Figure 2 A-C; (Krueger & Bear, 2011)), Tsc2+/- mice readily acquired this phase of the task, without making significantly more errors than WT littermate mice (see Figure 2 D-F). These data demonstrate that unlike FXS-model mice, under our experimental conditions Tsc2+/- mice are not impaired in either the acquisition or performance of the 5CSRTT.

In order to further assess whether Tsc2+/- mice may have a deficit in higher cognitive function we took an additional cohort of WT and Tsc2+/- mice (8-10 week old littermates) and assayed extinction in a two-choice discrimination task. In this series of experiments animals once again underwent food deprivation and daily habituation sessions in the 5CSRTT testing

apparatus. The testing apparatus was modified in order that only two nose-poke holes were accessible, one of which was illuminated. Animals were trained to nose-poke in the illuminated aperture in order to obtain a food reward. Once criterion responding was established (75% correct across five training days), extinction (five days) was commenced by no longer providing food reward following nose-pokes in the lit aperture. Once again we found that Tsc2+/- and WT mice exhibited comparable behavior, both showing extinction of nose-poking at a comparable rate (see Figure 3). This is in contrast with FXS-model mice that show exaggerated extinction as compared to WT control animals (see Figure 3). Together these findings suggest that Tsc2+/- mice may not exhibit deficits in higher cognitive function which are comparable to FXS-model mice. However it may also indicate that Tsc2+/- mice may have more subtle deficits in higher cognitive function that our behavioral assays are not yet sensitive enough to detect.

Specific aim 2: Characterization of visual cortical plasticity in $Tsc2^{+/-}$ mice and comparison of mGluR5 PAM and rapamycin treatment.

Therapeutically relevant insights have been gained by using the mouse visual cortex model in genetically defined developmental brain disorders such as Fragile X, Rett, and Angelman syndromes. Under this aim we will use well characterized visual assays (eg. ocular dominance plasticity) to characterize any deficits $Tsc2^{+/-}$ mice may have in cortical synaptic plasticity, and to assess the effect of PAM and rapamycin treatments on such impairments. These experiments have been initiated and we are currently assessing the visual abilites (acuity and contrast sensitivity) of $Tsc2^{+/-}$ mice to detrmine if they are comparable to that found in WT mice. Once characterized, we will proceed with experiments examining ocular dominance plasticity and stimulus-selective response potentiation (Exp 2.2).

Key Research Accomplishments:

1. Demonstrated that Tsc2+/- mice are amenable to food deprivation paradigms utilized to test higher cognitive function (s)
2. Demonstrated that $Tsc2^{+/-}$, unlike FXS model mice, show no deficits in the acquisition and performance of the five-choice serial reaction time task (5CSRTT).
3. Demonstrated that $Tsc2^{+/-}$ mice show no deficits in the extinction of a two-choice discrimination task.

Reportable Outcomes:

Talks & Presentations (by Dr. Mark Bear)

2012

Cold Spring Harbor Laboratory Seminar Series, January 5, 2012. Synaptic plasticity: from amblyopia to autism

Decadial Brain Symposium, Erasmus University, Rotterdam, The Netherlands, January 16, 2012

Frank Shobe Lecture in the Department of Psychiatry, Washington University School of Medicine, St. Louis, MO, March 6, 2012. Cortical synaptic plasticity: from amblyopia to autism

Mountcastle Lecture, The Johns Hopkins University School of Medicine, Baltimore, MD, March 23, 2012. Cortical synaptic plasticity: from amblyopia to autism

NINDS Grand Rounds, NIH, Bethesda, MD, March 27, 2012. Autism spectrum disorders: from synaptic pathophysiology to novel therapeutics

Disorders of Synaptic Dysfunction Symposium and Workshop, Baylor College of Medicine, Houston, TX, April 12-13, 2012. Fulfilling the promise of molecular medicine in fragile X

Ray Fuller Lecture, American Society for Pharmacology and Experimental Therapeutics Annual Meeting, San Diego, CA, April 25, 2012. Fulfilling the promise of molecular medicine in autism spectrum disorders

Simons VIP Workshop on 16p11.2 Deletions and Duplications, New York, New York, April 27, 2012

Alexandria Summit Neuroscience: Translating Innovation into New Approaches for Neuroscience, New York, New York, May 3-4, 2012. Moderator: Panel Discussion III - Understanding the neurodevelopment and exploring new approaches to treatment

ARVO 2012 Symposia Session: Learning to See: Experience Dependent Plasticity of the Visual System, Ft. Lauderdale, FL, May 7, 2012. Cortical synaptic plasticity and vision

University of California, San Francisco Neuroscience Formal Seminar Series, June 7, 2012. Cortical synaptic plasticity: from amblyopia to autism

Keynote Address, Gordon Conference, Fragile X and Autism-related Disorders, Stonehill College, June 10-15, 2012. Fulfilling the Promise of Molecular Medicine in Fragile X and Autism

4th Annual Tufts Neuroscience Symposium and William Shucart Lecture, October 4, 2012 Cortical Synaptic Plasticity: From Amblyopia to Autism

Mount Sinai Department of Psychiatry Grand Rounds, New York, New York, October 23, 2012. Fulfilling the Promise of Molecular Medicine in Fragile X and Autism

Brown University Neuroscience Seminar Series, December 13, 2012. Cortical Synaptic Plasticity: From Amblyopia to Autism

2013

Salk Symposium on Biological Complexity: Molecular Biology of Psychiatric Disorders, San Diego, California, January 16-19, 2013. Pathophysiology and treatment of fragile X and related disorders

Boston Children's Hospital Translational Medicine Symposium, February 5, 2013. Seaside Therapeutics and the science behind it: Forging a path from genes to treatments for autism spectrum disorders

AAAS Symposium, February 16, 2013. Session Title: From the Synapse to Brain Networks in Health and Disease

Keynote Address, Johns Hopkins Undergraduate Research Symposium. Brain Plasticity and Hippocampal Function

University of Pennsylvania, Department of Genetics and Department of Cell and Biology Seminar Series, April 8, 2013. Pathophysiology and treatment of fragile X and related disorders

Institute of Medicine Public Workshop – Accelerating Therapeutic Development of Nervous System Disorders towards First-in-Human Trials, April 8-9, 2013
Session IV: Accelerating Therapeutic Development-Preclinical Development

National Institutes of Health, National Institute of Neurological Disorders and Stroke, Curing the Epilepsies 2013: Pathways Forward, April 17-19, 2013 Session 5: What do new paths look like? Translation in Fragile X Syndrome

University of California, San Diego, Cellular and Molecular Medicine/Ludwig Institute for Cancer Research Seminar Series, April 25, 2013. Autism: From genes to pathophysiology to treatment

Conclusion:
Tuberous sclerosis complex (TSC) is a debilitating disorder that leads to significant disability for individuals and disrupt the lives of their families. Central nervous system disruption is one of the most pronounced features of the disorder, yet there is no treatment directed at the core neurological dysfunction that is definitive of TSC. In this research project, we have shown there is deficient mGluR5 function in Tsc2+/- mice, and proposed a novel method of treatment for the cognitive impairments associated with TSC by enhancing mGluR5 signaling with PAMs. Currently, the only potential therapy directed at the core disturbances in TSC is the inhibition of mTOR activity by the drug rapamycin. However, rapamycin has strong immunosuppressive properties, and therefore is not ideal for use as a chronic intervention treatment. The benefit of mGluR5 PAMs is that they do not share this detrimental property, and they target specifically the synaptic mechanisms that are likely responsible for the cognitive and behavioral impairments in TSC. Also, because mGluR5 PAMs do not directly activate or inhibit mGluR activity, but rather modulate the receptors' response to endogenous activation, they have the attribute of enhancing mGluR5 activity in a physiologically relevant way. We have demonstrated the potential of mGluR5 positive allosteric modulation as a treatment for TSC-related deficits in synaptic function and cognition and believe they may provide the TSC community with a novel, safe and effective treatment directed at the core central nervous system disruptions inherent to the disorder.

"So what section"
This work has profound implications for treatment of autism and intellectual disability. TSC and FXS represent two leading genetic risk factors for autism. Although great strides have been made in identifying genetic variation that correlates with non-syndromic autism, there is little known about autism pathophysiology—knowledge that is essential for developing effective therapies. Our test of the hypothesis that the Fmr1-/y and Tsc2+/- models of FXS and TSC have a shared synaptic pathophysiology has revealed that they are at opposite ends of a spectrum: the Fmr1 mutation causes exaggerated synaptic protein synthesis and LTD that are corrected by inhibition of mGluR5, whereas the Tsc2 mutation causes diminished synaptic protein synthesis and LTD that are corrected by augmentation of mGluR5. Moreover, the opposing effects of these mutations balance one another at synaptic and behavioral levels in the double mutant. This finding is interesting in light of recent discoveries that gain- and loss-of-function mutations in individual genes, such as MECP2, can often yield syndromes with overlapping features, such as epilepsy, cognitive impairment, and autism. Our findings reveal that even genetically heterogeneous causes of autism may produce similar deficits by bidirectional deviations from normal on a common functional axis. The important implication is that therapies designed to correct one cause of autism are not likely to be

effective for all other causes, and might well be deleterious. It will be critical to understand where a patient lies on the spectrum of synaptic function to choose an appropriate therapy for autism and other psychiatric disorders.

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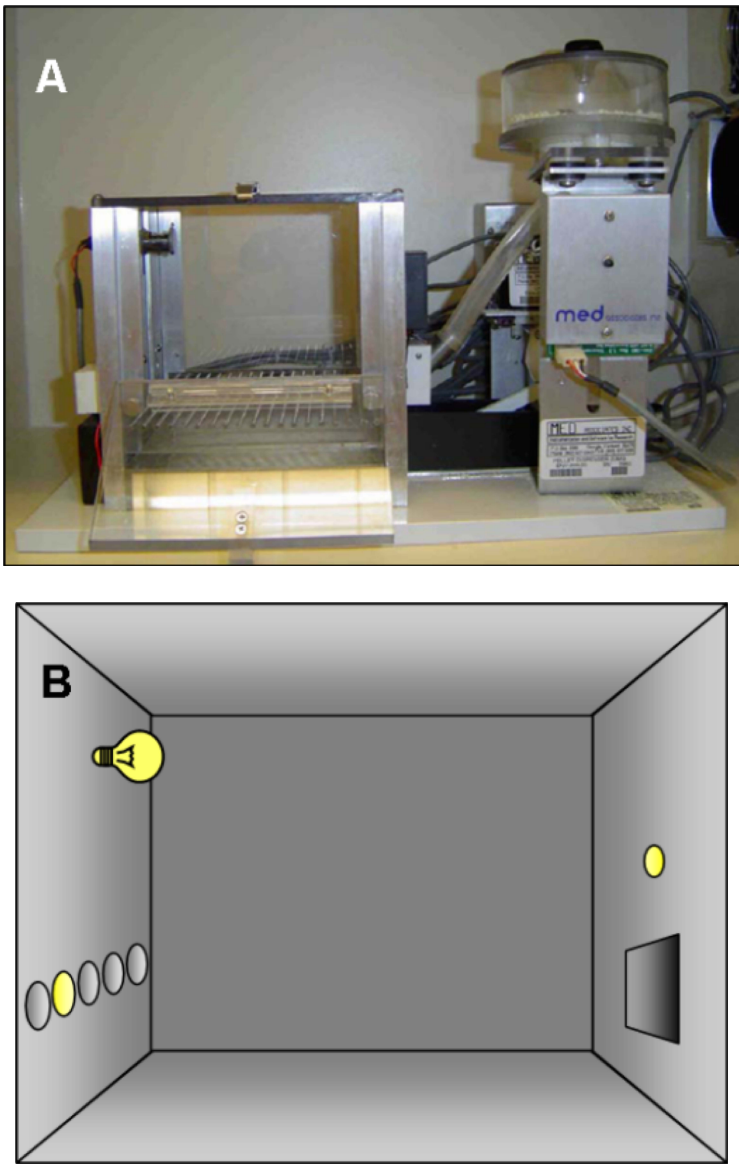

Figure 1. Photograph and schematic diagram of the 5 choice serial reaction time task (5CSRTT).
A. Photograph of the operant chamber used to assay higher cognitive function using the 5CSRTT. **B**. Schematic diagram showing the interior of the operant chamber. The chamber is equipped with five nose-poke holes (apertures that can detect a nose-poke response by the mouse) on the left wall and a food magazine (right wall) into which a food reward is delivered. In the experiments described, Tsc2+/- mice and WT littermates were trained to perform a nose-poke response in the illuminated aperture in order to obtain a food reward.

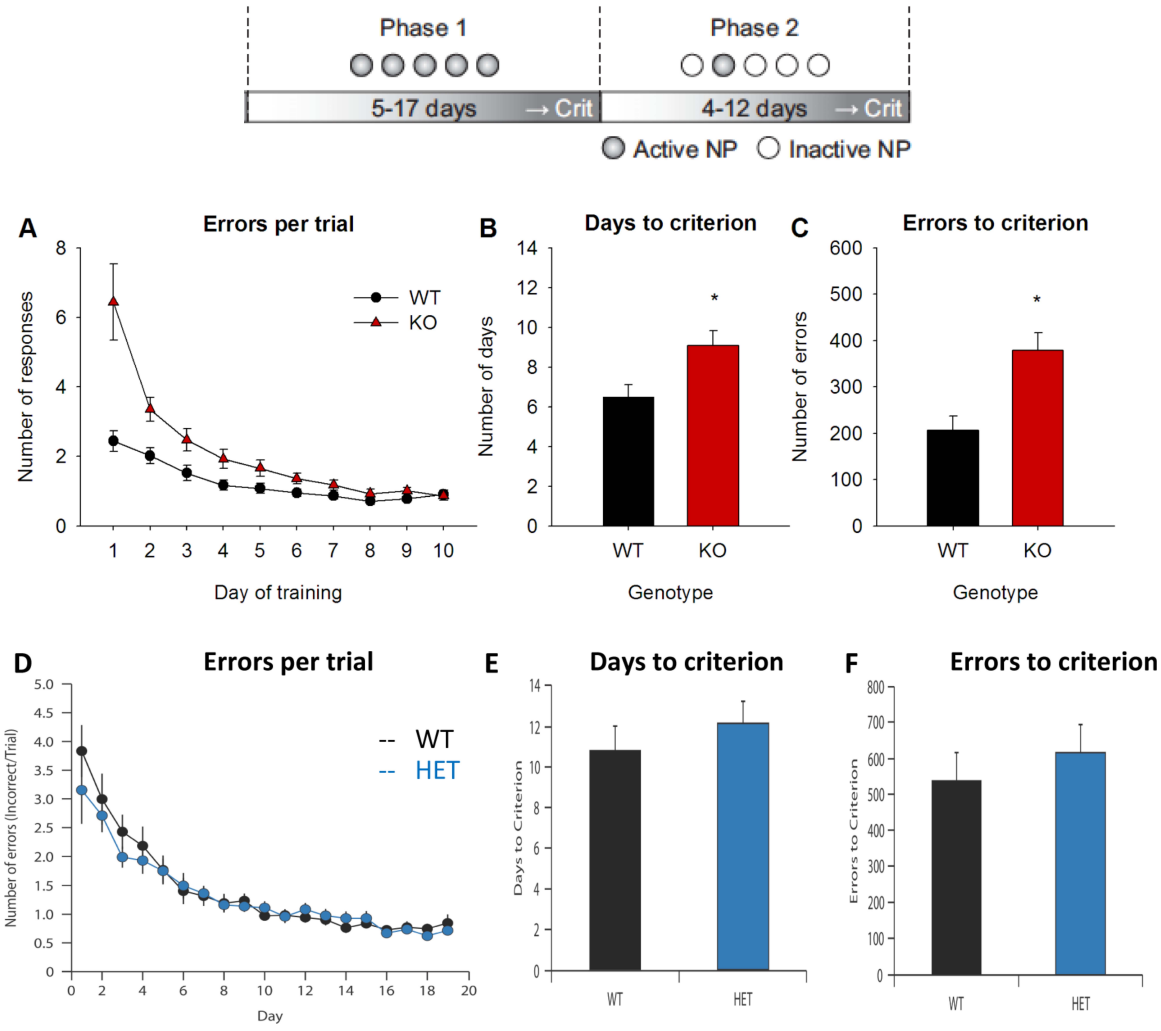

Figure 2. Tsc2+/- mice show no deficits in performance of the 5 choice serial reaction time task (5CSRTT). Top panel is a schematic diagram illustrating the design of the 5CSRTT. Following habituation and magazine training in the operant conditioning chamber, phase 1 was initiated in which all five nose-poke apertures were active (active NP) and food reward was provided following a nose-poke in any aperture. In phase 2, only one nose-poke aperture was active (active NP). The other four apertures were inactive (inactive NP) and were recorded as an "error" if the subject nose-poked. **A-C**. FXS model mice show an impairment in the 5CSRTT, as they exhibited significantly more errors across days in phase 2 training (**A**), they required more days to reach criterion (**B**), and made significantly more errors than their WT littermates (**C**). In contrast, Tsc2+/- mice exhibited similar performance to their WT littermates in: (**D**) the number of errors made across days to reach criterion, (**E**) the number of day to reach criterion performance and (**F**) the total number of errors made across days.

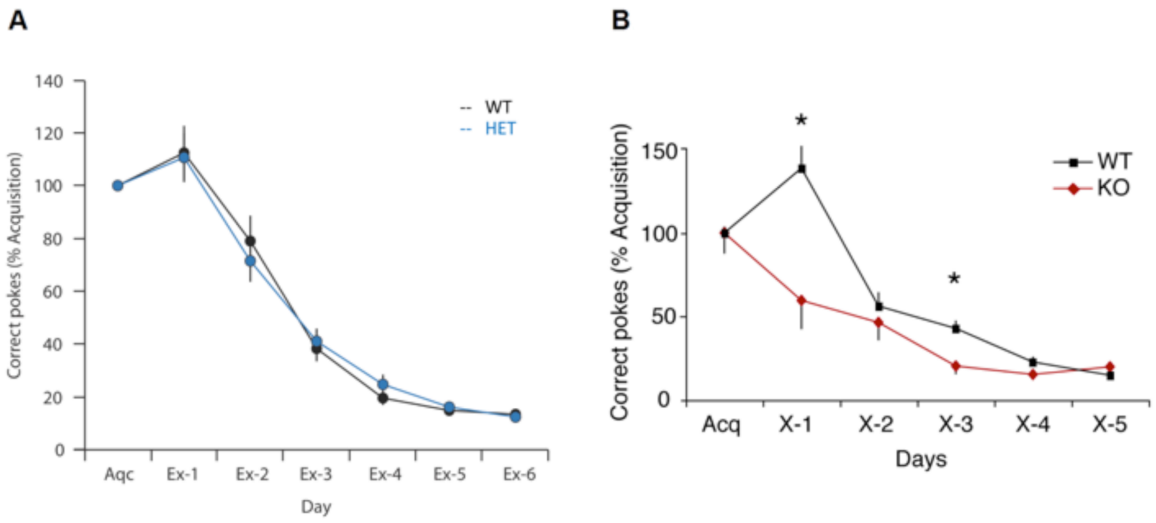

Figure 3. Tsc2+/- mice show no deficits in extinction in a two-choice discrimination task. **(A)** Tsc2+/- mice exhibit a similar rate of extinction to their WT littermates in a two-choice discrimination task. **(B)** Fmr1 KO mice show a more rapid rate of extinction as compared to their WT littermates. Data are expressed as a percentage of the performance during the last two training days (acq, acquisition; Ex, extinction). Asterisks indicate significance by post hoc t-tests ($p<0.05$).